EXHIBIT E

Acta Oto-Laryngologica, 2006; 126: 1012–1021

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

Immune-mediated inner ear disease

R. BOVO, C. AIMONI, & A. MARTINI

Department of Audiology, University of Ferrara, Ferrara, Italy

Abstract

The incidence of autoimmune inner ear disease (AIED) is difficult to determine: probably it is a rare disease, accounting for < 1% of all cases of hearing impairment or dizziness. Nevertheless, the diagnosis of AIED might be overlooked because of the lack of a specific diagnostic test. The hallmark of this clinically diagnosed condition is the presence of a rapidly progressive, often fluctuating, bilateral sensorineural hearing loss (SNHL) over a period of weeks to months. The progression of hearing loss is too rapid to be diagnostic for presbycusis and too slow to conclude a diagnosis of sudden SNHL. Vestibular symptoms, such as generalized imbalance, ataxia, positional vertigo and episodic vertigo may be present in almost 50% of patients. Occasionally only one ear is affected initially, but bilateral hearing loss occurs in most patients, with symmetric or asymmetric audiometric thresholds. Almost 25–50% of patients also have tinnitus and aural fullness, which can fluctuate. Systemic autoimmune diseases coexist in 15–30% of patients.

Keywords: *Immunology, cochlear disorders, vestibular disorders, hearing loss*

Introduction

The concept that the immune system may have a role in some idiopathic hearing loss was introduced during the early decades of the last century by Joannovic and Masugi [1,2]. In 1958 Lehnard [3] suspected that some cases of sudden bilateral hearing loss could be related to the production of anticochlear antibodies. Kikuchi [4], in 1959, wrote of 'sympathetic otitis', whereby surgery on one ear affected hearing in the other. He proposed autoimmune phenomena as the aetiology. In 1961 Beickert [5], and 3 years later Terayama [6] presented data supporting autoimmunity in experimental guinea pig cochleae. Different aetiological hypotheses related to immunological mechanisms were proposed in the subsequent years by other authors [7–9]. In 1960 Cody and Williams [10], while dealing with Cogan's syndrome, hypothesized that this syndrome should not be confined only to the cornea and VIII cranial nerve, but should probably be a part of a generalized disease, characterized by vascular alterations and identifiable as a collagen disease. In 1979 McCabe [11] first described a cohort of 18 patients with bilateral asymmetric hearing loss progressing over weeks to months which responded to steroid therapy. He proposed the definition of autoimmune hearing loss and asserted the importance of a high index of suspicion in these patients since, if diagnosed early, they could be treated and their hearing preserved. His work stimulated research into this topic. At the same time Harris and Ryan [12] recommended caution in determining the relationship of immunological phenomena to ear disease aetiology. Based on the immunological criteria published by Rose and Bona [13], Gloddek and Arnold [14] suggested that the diagnosis of an autoimmune disease relies on three levels of proof which are, in descending order of importance: (i) direct proof; (ii) indirect proof; and (iii) circumstantial evidence. Direct proof means the induction of disease in humans by transferring autoantibodies or autoreactive T cells, which is ethically unacceptable. Indirect proof derives from the induction of autoimmunity in animal models, using transfer of autoantibodies or reactive cells. As stressed by Yoo and Yazawa [15], experimental animal diseases and immunopathological manifestations in the natural human should parallel each other. Circumstantial evidence refers to: (i) the

Correspondence: Roberto Bovo, Department of Audiology, Arcispedale S. Anna, Corso Giovecca, 203 IT-44100 Ferrara, Italy. E-mail: bovoroberto@libero.it

ISSN 0001-6489 print/ISSN 1651-2551 online
DOI: 10.1080/00016480600606723

association with other autoimmune disease, (ii) lymphocytic infiltration of the target organ, which is impossible to access because the inner ear is not amenable to diagnostic biopsy, (iii) genetic restriction to hearing loss and autoimmune disease and (iv) response to immunosuppressive therapies [14]. Nowadays experimental models of autoimmune hearing loss have been developed in a variety of animals [14,16,17]. The correlation between experimental models in animals and serological findings in human might be significant [18]. Several studies seem to demonstrate that genetically controlled aspects of the immune system may increase or otherwise be associated with increased susceptibility to different inner ear diseases [19]. Moreover, autoimmune activity in patients with idiopathic hearing loss has been assessed by the migration inhibition test [11], enzyme-linked immunosorbent test (ELISA) [20,21], lymphocyte transformation test [22,23], immunofluorescence microscopy [23,21] and immunoblotting [24,25].

A wide variety of antibodies against different ear tissue have been detected. However, the recognition of multiple antigens did not identify any one as being the specific target in autoimmune inner ear disease (AIED) [17]. As suggested by Soliman [26], the large variety and variability of the observed inner ear antibodies does not always support the cause-and-effect link needed to demonstrate the correlation between antibodies and inner ear disease. However, based on all these clinical and experimental data there is today strong evidence of immune mechanisms in human inner ear disease.

The immune system of the inner ear

Historically the brain was thought to be protected from immune response by the blood–brain barrier, and the inner ear was considered an immune-privileged site. Since then evidence has been accumulating that both the brain and the inner ear are involved in processes of immune surveillance and action. The inner ear has an immune system with immunocompetent cells and trafficking of antibodies [12].

The hypothesis that the endolymphatic sac (ES) might have a predominant role in the local immune response was first proposed by Lim and Silver in 1974 [27].

Once the inner ear has been activated by antigenic stimuli different immunocompetent cells seem to develop in the ES or to mobilize from systemic circulation penetrating the blood labyrinthine barrier. The presence of macrophages, B cells and T cells in the ES and in the perisaccular space has been demonstrated by several authors in humans and animals [28–30].

Several studies using immunohistochemical methods have been concerned with the anatomical localization of the different immunocomponent cells. T helper (CD4) cells predominate in the ES, while CD8 cells are normally scanty, but the relationship between T-helper CD4 and CD8 cells may be inverted in case of chronic antigen stimulation as in the presence of acoustic neuroma [30]. Infiltration and trafficking of leukocytes in the inner ear has been largely investigated as an important feature of immune and inflammatory processes, that in most unfavourable cases can finally result in cochlear fibrosis or osteogenesis. The blood vessels of the spiral modiolar vein (SMV) adjacent to the scala tympani are the initial site through which lymphocytes enter the inner ear, in response to different stimulation: e.g. viral infection, acoustic trauma or vascular damage. As early as 6 h post-stimulation cells accumulate around and within the SMV and then began to stream into the scala tympani along the bony canaliculi containing the collecting venules [31].

The control of lymphocyte entry into the inner ear is not well understood. The ES is probably critical in lymphocyte recruitment signals to the labyrinth. Tomiyama and Harris [32] demonstrated that oblation of the ES reduces immune responses in the cochlea. A reaction of ES lymphocytes to antigens probably determines the release of a diffusible mediator, perhaps an interleukin. In fact, interleukin-2 (IL-2) is not present in the perylimph in the resting state, but is produced during immune responses.

The peak rise of IL-2 in the perilymph is reached at 18 h following antigen stimulation, and declined over a 5-day period [33], well in accordance with the entry of helper T cells and macrophages. Furthermore, another mediator such as intercellular adhesion molecule (ICAM-1) reaches a maximum level on the epithelium of the SMV and collecting venules by day 2 and then gradually reduces. Yeo et al. [34] demonstrated that antigens in the scala tympani may flow to the ES, where they may be presented by macrophages to the systemic immune system.

As regards immunoglobulin, it has been demonstrated that predominantly IgG, with lesser amounts of IgM and IgA, are present in the perilymph of humans and in experimental animals [35], but their origin is still debatable. Mogi et al. [36] demonstrated that immunoglobulin in the perilymph might come largely from perilymphatic blood vessels rather than from the cerebrospinal fluid.

Autoimmune inner ear disease (AIED)

Viral infection, trauma, vascular damage and immune mechanisms are all involved in the aetiopathogenesis of inner ear damage. An immune-mediated reaction does not always have negative effects: systemic immunization with viral antigens may protect the inner ear from a later viral infection [37]. However, as inner ear cells are delicate and have limited abilities of repair and regeneration, immunity should be closely regulated. A disruption of regulating mechanisms may cause substantial damage to the inner ear structures, as demonstrated either in animal models or in human diseases. There is today substantial evidence of autoimmune mechanisms in some of the inner ear disease entities, including Ménière's disease, otosclerosis, progressive sensorineural hearing loss (PSHL) and sudden deafness. Furthermore, in several systemic autoimmune diseases the vestibulo-cochlear system may be affected. Yoo and Yazawa [15] identified lists of ear diseases with probable immunological features (Table I) and autoimmune diseases affecting hearing (Table II). The clinical features of these conditions may be very different regarding unilaterality or bilaterality, characteristics of onset, ratio of auditory versus vestibular involvement, rate of progress and response to therapy [38]. An increasing body of experimental studies has investigated the role of cellular, humoral and/or genetic factors. Probably the inner ear response is basically cellular, which has been suggested in several animal models, with recruitment of inflammatory cells toward the cochlea. There is clear evidence that the chronic activation of helper T lymphocytes reactive against self-proteins is the mechanism that maintains the destructive autoimmune process. Why this occurs is still debatable.

Table I. Ear diseases with immunological features.

Region of ear	Disease
External ear	Auricular chondritis
	Relapsing polichondritis
Tympanic membrane	Tympanosclerosis
Eustachian tube	Autoimmune salpingitis
Middle ear	Otosclerosis
	Secretory otitis media
	Necrotizing otitis media
	Cholesteatoma
Inner ear	Autoimmune sensorineural hearing loss
	Ménière's disease
	Otosclerosis
	Cochlear vasculitis
	Sudden hearing loss
Retrocochlear	Autoimmune central nervous system disease

Table II. Autoimmune diseases affecting hearing.

Relapsing polychondritis
Systemic lupus erythematosus
Disseminated vasculitis
Rheumatoid arthritis
Sjögren's syndrome
Systemic sclerosis
Myasthenia gravis
Hashimoto's thyroiditis
Goodpasture's syndrome
Vogt-Koyanagi-Harada syndrome
Cogan's syndrome
Behçet's disease
Sarcoidosis
Wegener's granulomatosis

The 'cross-reactions' theory is presently the most favoured: antibodies or rogue T cells cause accidental inner ear damage because the ear shares common antigens with a potentially harmful substance, virus or bacteria that the body is fighting off. Another mechanism is involved in the so-called sympathetic cochleolabyrinthitis that has been reproduced in animal models [14]. In the eye there is an analogous syndrome, 'sympathetic ophthalmia', where following a penetrating injury to one eye, the other eye may go blind. As a consequence of viral infection, acoustic or physical trauma, vascular damage or operative intervention of the inner ear, lymphocytes become sensitized as a result of exposure to proteins in the damaged cochlea. Under normal conditions these anatomically sequestered proteins are recognized as 'foreign' and serve as an antigen, resulting in the sensitization of lymphocytes. These cells recirculate as 'memory' lymphocytes – the donor cells in the animal experiment – and reach the intact contralateral cochlea. This results in an immune response and destruction of the organ. The induction of disease in humans by transferring autoantibodies or autoreactive T cells is ethically unacceptable and the ear is not amenable to diagnostic biopsy. For these reasons experimental animal models have been widely employed in attempts to verify the mechanisms of autoimmune hearing loss. Injection of antigen into an animal that has not been previously sensitized produces modest, transient, or no hearing loss and minimal inflammation [39]. Secondary immune response animals develop much higher antibody levels than primary animals. The associated inflammation can lead to substantial damage in the inner ear and moderate to severe hearing loss [40,41]. This is not seen in control animals immunized with proteins from another tissue. These models have been developed using the following antigens: type II collagen [42] type IX collagen [43], Po protein [44] and tubulin [45].

A wide variety of antibodies against different ear tissues has been studied in the last two decades: antibodies to 68–72 kDa inner ear antigen have been detected in the sera of affected individuals [24,46–48]. Patients with positive test results for these antibodies were more likely than those with negative results to have hearing loss that responded to immunosuppressive therapy [46,47]. In the same year two groups [49,50] reported that the 68-kDa protein seemed to be heat shock protein 70 (hsp 70). Heat shock proteins are constitutively produced by host and pathogens and usually are up-regulated in response to infection or other stresses, including heat, ischaemia and toxic agents. However, since then hsp 70 has not held up as a high-probability inner ear target antigen and in recent years this hypothesis has been proved wrong by experimental data [51–53].

Thomas Carey's group developed monoclonal antibodies to cells isolated from the guinea pig organ of Corti and observed that mice carrying the Kresge Hearing Research Institute-3 (KHRI-3) antibody developed hearing loss [54]. In the guinea pig organ of Corti, the KHRI-3 antibody binds to supporting cell with a distinctive 'wine glass' pattern. Patients with suspected AIED often have antibody that binds to guinea pig inner ear supporting cells with the same wine glass pattern [48]. Furthermore, infusion of purified antibody into the cochlea yields in vivo binding to supporting cells in the organ of Corti that is accompanied by loss of outer hair cells, loss of hearing, and redistribution of the antigen in the regions of hair cell loss [54,55]. Recently, Carey's group [53] has isolated the guinea pig inner ear supporting cell antigen (IESCA) and sequenced it by mass spectroscopy, demonstrating that it contains multiple peptides identical to those in human 'choline transporter-like protein 2' (CTL2). The overall CTL2 sequence in guinea pig and humans diverges, as expected for species that are evolutionarily distant from one another, but for the most part the sequences are identical at DNA (86%) and protein (90%) levels, suggesting that the function is the same in both species [53]. Choline is required either for biosynthesis of acetylcholine, which is an important neurotransmitter in the inner ear, or for biosynthesis of phosphatidylcholine, a major component of membranes necessary for all cells. Most probably CTL2 complex provides something to supporting cells that is essential for hair cell survival. In conclusion, there is a mounting evidence that the target antigen of 68 kDa antibody is not the hsp 70 (as believed for the last 15 years), but the CTL2 protein.

There is evidence for cytokines in the cochlea including IL-1A, tumour necrosis factor (TNF)-α, NFkB P65 and P50, and IkBa [56]. In general, Th1-type cytokines (interferon-γ, IL-2) perpetuate the inflammatory response to autoimmunity lesions, whereas Th2-type cytokines (IL-4, IL-10, etc.) seem to control the inflammatory response.

As regards genetic factors there is evidence that genetically controlled aspects of the immune system may increase or otherwise be associated with increased susceptibility to common hearing disorders such as Ménière's disease (MD). Bernstein and associates [57] reported that 44% of patients with MD, otosclerosis and striatal presbyacusis had one particular extended MHC haplotype (Dqw2-Dr3-c4Bsf-C4A0-G11: 15-Bf:0.4-C2a-HSP70:7.5-TNF), compared with only 7% of controls. Sudden hearing loss in Koreans that does not recover is also associated with HLA-DRB1*04, DQA1 03 and 05 [58]. Melchiorri et al. [59] found a strong increase in the frequency of the HLA-Cw*07 alleles in MD patients (63.4%) when compared with healthy controls (35.6%) or with patients affected by other internal ear diseases (32.3%). On the other hand, a recent study by Lopez-Escamez and others [60] performed in Spain found no difference in HLA antigens between 54 patients with definite MD and normal controls. The genetic background of HLA studies is important and it is possible that one group might find HLA differences which are not found in another. These data are thus conflicting. If there is indeed an association with HLA, at least in certain populations, it would suggest that more of MD and other progressive syndromes may be caused by immune dysfunction than is generally considered at present. It is important to remember that HLA typing is relevant when considered in the context of the patient's genetic background. In other words, results of studies of Korean subjects for example, such as reported by Yeo et al. [58], may not apply to persons of non-Korean ethnicity.

Recently new gene products, expressed by CD4+ T cells during the induction of the state of anergy, have been identified [61]. By studying their potential role in the maintenance of tolerance induced by immunotherapy, it might be possible to develop new therapeutic strategies.

In the pathogenesis of AIED, there is clear evidence that collagen represents one of the most important antigens. In 1982 Yoo et al. [62] demonstrated a raised level of serum antibodies to bovine type II collagen in 5 of 12 patients with otosclerosis. In the following years several authors reported the association between type II collagen antibodies and different inner ear diseases such as MD, otosclerosis, idiopathic progressive sensorineural hearing loss (PSNHL) and sudden deafness [63,64]. Furthermore there is experimental evidence

that the autoimmune inner ear disease provoked in animals by these antibodies closely resembles the AIED in humans [65–67]. Direct infusion of monoclonal antibody against CB11 peptide in the cochlea produces endolymphatic hydrops [67]. Yoo et al. were successful in producing ear lesions by transferring, through serum exchange, collagen type II-induced antibodies from immunized rats to normal recipients. Type II collagen autoimmunity has also induced hearing loss and imbalance in non-human primates [68].

MD has been ascribed to different causes, including immune-mediated or even autoimmune mechanisms. The last hypothesis is supported by many experimental data: (1) hydrops can be induced experimentally by injection of antigens or monoclonal antibodies; (2) the presence of antibodies to inner ear antigens, e.g. type II collagens, 30 kDa protein, c-Raf, β-tubulin, 68 kDa protein has been demonstrated; (3) the deposition of circulating immune complexes may produce inflammation and interfere with the sac's filtering capability; (4) antiviral antibodies and lymphocyte blastogenesis have all been demonstrated; (5) certain D-related loci may be associated with MD; (6) the endolymphatic sac is the site of the immune response of the inner ear and is also the site mostly involved in MD pathogenesis; it may be a target of mediators released from systemic inhalants or food reactions; (7) the temporal bone changes are associated with immunological changes; (8) efficacy of steroid treatment.

Incidence and clinical features

Incidence of AIED is difficult to determine and controversial: probably it is a rare disease, accounting for $<1\%$ of all cases of hearing impairment or dizziness. Nevertheless, the diagnosis of AIED might be overlooked because of the lack of a specific diagnostic test. The disease seems more common in female than in male patients and most often initial onset of symptoms occurs between 20 and 50 years of age. Females between the ages of 17 and 42 years represent 65% of the cases reported by Hughes et al. [22]; 20% of the patients in this study later manifested signs of systemic autoimmune disease. Regarding MD, it seems possible that about 16% of bilateral cases and 6% of any other cases of this disease may be due to immune dysfunction.

The hallmark of this clinically diagnosed condition is the presence of a rapidly progressive, often fluctuating, bilateral SNHL over a period of weeks to months. The progression of hearing loss is too rapid to be diagnostic for presbycusis and too slow to conclude a diagnosis of sudden SNHL. Vestibular symptoms, such as generalized imbalance, ataxia, motion intolerance, positional vertigo and episodic vertigo may be present in almost 50% of patients. In the McCabe series [11] two-thirds of the patients had low grade vestibular symptoms without spells. Occasionally only one ear is affected initially, but bilateral hearing loss occurs in most patients (79%), with symmetric or asymmetric audiometric thresholds. Almost 25–50% of patients also have tinnitus (ringing, hissing, roaring) and aural fullness, which can fluctuate. Systemic autoimmune diseases (Table II) coexist in 15–30% of patients, occasionally affecting the external ear skin. Facial palsy, as well as tissue destruction of the tympanic membrane, middle ear and mastoid may also occur. Nevertheless, the physical examination of the ear is usually normal.

Laboratory studies

While specific tests for autoimmunity to the inner ear would be desirable, at the time of writing there are none that are both commercially available and proven to be useful. Antigen-specific tests include: migration inhibition test (MIT), lymphocyte transformation test (LTT) and Western blot analysis for antibodies to inner ear antigen. MIT is a gross test for cell-mediated immunity and was first used by McCabe [11]. Its use is limited because of inherent technical difficulties. The LTT measures the response of the patient's sensitized lymphocytes to known inner ear antigens. The patient's lymphocytes are exposed to serum containing inner ear antigens. A proliferative response occurs and is compared to that of known negative control lymphocytes. Measurement is made by recording the incorporation of tritiated thymidine into new DNA as cell synthesis occurs. This test was first used by Hughes et al. [22] in patients with idiopathic PSNHL. Results are still controversial. Berger et al. [69] demonstrated positive results in half of the 68 patients with PSNHL, and Hughes et al. [70] found test positivity in 19% of their patients. On the other hand Harris and Sharp [24] found no significant difference between the patients and the control group.

A commercially available test, called 'anti-68 kD (hsp 70) Western blot' was reported to detect a local autoimmune inner ear process in the absence of any systemic autoimmune process and to be correlated with steroid responsiveness [46]. The test uses purified hsp 70 kDa antigen from bovine kidney cell line and is based on the assumption that the 68 kDa protein is the hsp 70. Unfortunately, as discussed above, this assumption proved to be wrong. Furthermore, the sensibility reported in different studies is either very low (22% in a study by Gottschlich et al. [47]) or positivity is

similar in patients with clinical AIED and the normal population [51,71].

At present it is generally felt that LTT and anticochlear antibody blood tests are not useful.

Identification of antibody to an inner ear supporting cell antigen with immunofluorescence (IF) microscopy [21,23] of guinea pig cochlea may have value in the diagnosis of patients with AIED, although not in current clinical practice. This technique has been shown to be more sensitive and specific than Western blot [72,73]. Patients with IF-positive serum are nearly three times more likely to experience improved hearing with corticosteroid treatment than those who are IF-negative [72]. For the moment, diagnosis of AIED is generally based either on clinical criteria, or a positive response to steroids, or evidence from broader tests of autoimmunity. A non-specific antigen screening test is useful for evidence of systemic immunologic dysfunction, yet does not strictly correlate with a diagnosis of immune-mediated inner ear disease. In clinical practice the antigen-non-specific tests usually recommended are as follows. (1) Blood tests for autoimmune disorders: levels of circulating immune complexes, sedimentation rate, ANA, Raji cell, rheumatoid factor, complement C1Q, smooth muscle antibody, TSH and anti-microsomal antibodies, anti-gliadin antibodies (for coeliac disease), HLA testing. (2) Blood tests for conditions that resemble autoimmune disorders: FTA (for syphilis), Lyme titre, HBA1C (for diabetes, which is often autoimmune-mediated), HIV (HIV is associated with auditory neuropathy).

Brookes [74] described raised levels of circulating immune complexes (CIC) in patients with PSNHL, sudden hearing loss and MD. Other studies demonstrated raised CIC levels [75,76].

Furthermore, Brookes [74] also reported that C3c and C1q levels are significantly raised in MD;

27 (45.8%) of 59 patients with MD had at least 1 serum autoantibody, compared with 8 (27.6%) of the 29 controls ($p < 0.02$). The most common autoantibodies were antinuclear antibody in 17 patients, antithyroglobulin antibody in 9 patients and anti-smooth muscle antibody in 9 patients. Also in patients with PSNHL the incidence of antinuclear and antithyroid antibodies was high, whereas the same antibodies were absent in patients with sudden hearing loss.

Medical therapy for AIED

Treatment for AIED seems to be changing rapidly and new protocols are constantly being attempted. As the natural history of untreated immune-mediated inner ear disease is unknown the efficacy of these protocols is evaluated on empirical clinical data gathered during the last two decades. The mainstay of this treatment is the positive response to anti-inflammatory drugs, particularly corticosteroids, in term of improved hearing. In McCabe's clinical study [11] and in several subsequent ones [63,77] the efficacy of immunosuppressive therapy is well demonstrated in a vast subset of patients. In cases with a classic rapidly progressive bilateral hearing impairment, an initial treatment with high-dose steroids (prednisone or dexamethasone) is generally tried, assuming that there are no contra-indications such as peptic ulcer disease, diabetes, glaucoma, hypertension or history of tuberculosis. These cases are analogous to rapidly progressive glomerulonephritis in that inner ear inflammation progresses to severe, irreversible damage within 3 months of onset (and often much more quickly). Thus, these patients are treated with a sense of urgency. A standardized regimen for steroid therapy does not exist, yet many recommend prednisone, 1 mg/kg/day for 4 weeks followed by a slow taper over several weeks to a maintenance dose of 10–20 mg/day or every other day. Shorter-term or lower dose long-term therapy has either been ineffective or appears to increase the risk of relapse. Patients often learn what maintenance dose is necessary to preserve hearing as disease activity often waxes and wanes. If hearing suddenly worsens or tinnitus reappears in one or both ears during the taper it is an indication to repeat the initial high dose treatment. Nevertheless, not all patients respond to corticosteroid therapy in the same manner: some demonstrate improvement in threshold, discrimination scores or both, while others have fluctuation without a definite improvement and still others progress to profound hearing loss despite aggressive immunosuppressive treatment. Steroid treatment is inexpensive, but in some patients it is difficult to maintain because of side effects or because hearing loss becomes refractory to them. In most cases of patients with no response to steroids within 6–8 weeks, a cytotoxic chemotherapy type of medication such as methotrexate (MTX) and cyclophosphamide has been used over the long term [78,79]. These agents are associated with considerable toxicity and adverse effects: myelosuppression, haemorrhagic cystitis, infertility and increased risk of malignancy. Furthermore, a recent multicentric study has demonstrated that MTX was no more effective than placebo in maintaining the hearing improvement achieved with prednisone treatment [77]. When dealing with cytotoxic drugs the following consultations are recommended: rheumatologic, immunologic and haematological. Furthermore, these consultations in addition with ophthalmologic and neurological

ones are useful in ruling out systemic diseases. As suggested by Ryan et al. [80] it is questionable whether every suspicious case should be treated with these powerful drugs: the decision regarding when and how to treat should always be multidisciplinary. The normal oral dose of MTX is 7.5–20 mg weekly with folic acid, and with this dosage MTX seems to be less toxic and to be associated with a lower risk of neoplasia in respect to cyclophosphamide. However, the patient needs to be regularly monitored by means of complete blood count, platelet count, determination of urea nitrogen and creatinine levels, liver function tests and urinalysis. Cyclophosphamide in addition to steroids has been recommended by McCabe [11] as a first-line treatment with the following regimen: intravenous cyclophosphamide 5 mg/kg per day for 2 weeks, followed by a rest period of 2 weeks, and than a final 2 weeks of infusions; in addition dexamethasone 16 mg/day orally for 2 months, followed by tapering to 2–4 mg over 2 months. Steroids are continued for 6–24 months, depending on symptoms. Liberal amounts of fluid intake are recommended and again peripheral blood counts at regular intervals. Administration in children and young people must be avoided because of the risk of permanent sterility. If speech discrimination scores increase by 20% or pure tone average improves by 15 dB, therapy is continued for 3 months. Cyclophosphamide is tapered first followed by steroids. If symptoms recur both drugs are restarted. Three-month cycles are continued until patients can be weaned. No patient required more than 24 months of treatment in McCabe's study [11]. In 1989 Luetje and Berliner [81] introduced the use of plasmapheresis in patients with AIED. Improvement of auditory function occurred in six of eight patients, three of whom were able to discontinue immunosuppressive medication. In plasmapheresis blood taken from patients is centrifuged to remove the plasma with antibodies, antigens, immune complexes and other immune mediators. Cells are saved and resuspended in normal fresh plasma or albumin in saline and returned to the patients. Plasmapheresis is expensive and is only suitable for disorders mediated by antibodies or immune complexes. Treatments are given three times weekly for 2 weeks followed by once weekly for 4 additional weeks. Although it may reduce the autoantibody's effects by 65% by removing approximately 2500 ml of plasma, it is actually considered an adjunctive therapy. Hughes advises plasmapheresis for those patients who are unresponsive to steroids and cytoxan after 6–8 weeks at the doses stated above.

Antagonists against proinflammatory cytokines, such as IL-1 and TNF have being tested. Among them, etanercept (Enbrel), a TNF receptor blocker drug, initially seemed to demonstrate good efficacy [82,83], but in new trials the results were no better than placebo [84,85]. Etanercept 25 mg is given as an injection twice a week for 8 weeks; presently it is very expensive and in short supply and its application remains to be further investigated. Immunosuppressive drugs like cyclosporine A and FK 506 are also used to block T-cell activation. Several experimental therapies have been studied. In animals, attempts have been made to induce tolerance by administration of antigens that cause autoimmunity or peptide competition: variants of AIED have been treated with oral collagen [86]. Oral tolerization to antigen has been applied to autoimmune disease patients, although the exact mechanisms involved are still not clear. Most probably active suppression and clonal anergy or clonal deletion are the main mechanisms of oral tolerance. Several antigens have been given orally to patients, such as bovine myelin basic protein (MBP) to patients with multiple sclerosis, or type II collagen to patients with rheumatoid arthritis. For these reasons, the identification of autoantigens in AIED is of paramount importance for better management of these patients. Monoclonal antibodies against surface antigens (e.g. CD3) or the T-cell receptor might be used to block lymphocytes. Local application of steroids in animal middle ear led to higher and more sustained perilymph levels than systemic treatment [87]. In humans, however, round window application of different immunosuppressive drugs via an osmotic mini-pump was ineffective in reducing immune-mediated hearing loss [88]. It is reasonable to suspect that while systemic treatment reduces circulating leukocytes and lymphocytes, local treatment is unable to reduce the entry in the middle ear of these cells, which may become irresponsive to treatment, once highly activated in the ear. The aim of local treatment should be a blockage of immune and inflammatory cells trafficking into the inner ear. ICAM-1 is a molecule present on activated endothelial cells that recruits lymphocytes into the inner ear [89]. Intravenous anti-ICAM-1 has been proven to reduce the recruitment of leukocytes into the scala tympani, the VIIIth nerve and the perivascular spaces around the vessels of the SMV and modiolar veins [90].

References

[1] Joannovic D. Zur Wirkung fermentativ gewonner spaltungsprodukte aus Geweben und Bakterien. Wein Klin Wochenschr 1920;70:1410–1.

[2] Masugi M, Tomizuka Y. ber die spezifisch zytotoxischen Veränderungen der Niere und sehr Leber durch das spezifische Antiserum (Nephrotoxin und Hepatoxin). Trans Jap Pathol 1931;21:329–41.

[3] Lehnhardt E. Plotzliche horstorungen auf beiden seiten gleichzeitig oder nacheinander aufgetreten. Z Laryngol Rhinol Otol 1958;37:1.
[4] Kikuchi M. On the "sympathetic otitis". Zibi Rinsyo Kyoto 1959;52:600.
[5] Beickert V. Zur Frage der empfindungs Schwerhörigkeit under Autoallergie. Z Laryngol Rhinol Otol 1961;40:837–42.
[6] Terayama Y, Sasaki U. Studies on experimental allergic (isoimmune) labyrinthitis in guinea pigs. Acta Otolaryngol (Stockh) 1964;58:49–64.
[7] Schiff M, Brown B. Hormones and sudden deafness. Laryngoscope 1974;84:1959–81.
[8] Quick C. Antigenic cause of hearing loss. Otolaryngol Clin North Am 1975;8:385.
[9] Arnold W, Weidaueer H, Seelig H. Experimentellerbeweiß einer gemeinsamen Anti-genizitat zwischen Innenohr und Niere. Arch Otorhinolaryngol 1976;212:99.
[10] Cody D, Williams H. Cogan's syndrome. Laryngoscope 1960;70:447–78.
[11] McCabe B. Autoimmune sensorineural hearing loss. Ann Otol 1979;88:585–9.
[12] Harris J, Ryan A. Immunobiology of the inner ear. Am J Otolaryngol 1984;5:418–25.
[13] Rose N, Bona C. Defining criteria for autoimmune diseases (Witebski's postulates revisited). Immunol Today 1993;14: 426–30.
[14] Gloddek B, Arnold W. Clinical and experimental studies of autoimmune inner ear disease. Acta Otolaryngol Suppl 2002;(548):10–14.
[15] Yoo TJ, Yazawa Y. Immunology of cochlear and vestibular disorders. In: Luxon L, editor. Audiological medicine – clinical aspects of hearing and balance. London: Taylor & Francis; 2003. p. 61–87.
[16] Soliman A. Immune-mediated inner ear disease. Am J Otol 1992;6:575–9.
[17] Hefeneider SH, McCoy SL, Hausman FA, Trune DR. Autoimmune mouse antibodies recognize multiple antigens proposed in human immune-mediated hearing loss. Otol Neurotol 2004;25:250–6.
[18] Soliman A. Experimental autoimmune inner ear disease. Laryngoscope 1989;99:188–93.
[19] Bernstein JM, Shanahan TC, Schaffer FM. Further observations on the role of the MHC genes and certain hearing disorders. Acta Otolaryngol (Stockh) 1996;116:666–71.
[20] Yoo T, Kang A, Stuart J, Tomoda K, Townes A, Dixit S. Type II collagen autoimmunity in otosclerosis and Ménière's disease. Science 1982;217:1153–5.
[21] Plester D, Zanetti F, Timpl R, Berg P, Klein R. Diagnostic laboratory tool for immune-mediated sensorineural hearing loss. In: Veldman JE, editor. Immunobiology, histophysiology and tumor immunology in otolaryngology. Amsterdam: Kugler Publications; 1988. p. 33–7.
[22] Hughes G, Kinney S, Barna B, Calabrese L, Hamid M. Autoimmune reactivity in Ménière's disease: preliminary report. Laryngoscope 1983;43:410–7.
[23] Arnold W, Pfatz R, Altermatt H. Evidence of serum antibodies against inner ear tissue in the blood of patients with certain sensorineural hearing disorder. Acta Otolaryngol (Stockh) 1985;99:437–44.
[24] Harris J, Sharp P. Inner ear autoantibodies in patients with rapidly progressive sensorineural hearing loss. Laryngoscope 1990;100:516–24.
[25] Cao M, Gersdorff M, Deggouj N, Warny M, Tomasi J. Detection of inner ear disease autoantibodies by immunoblotting. Mol Cell Biochem 1995;24;146:157–63.
[26] Soliman A. Autoantibodies in inner ear disease. Acta Otolaryngol (Stockh) 1997;117:501–4.
[27] Lim D, Silver P. The endolymphatic duct system. A light and electron microscopic investigation. In: Pulec J, editor. Barany Society Meeting. Los Angeles; 1974. p. 390.
[28] Rask-Andersen H, Stahle J. Immunodefence of the inner ear lymphocyte-macrophage interaction in the endolymphatic sac. Acta Otolaryngol (Stockh) 1980;89:283–94.
[29] Harris J. Immunology of the inner ear: evidence of local antibody production. Ann Otol Rhinol Laryngol 1984;93: 157–62.
[30] Alternatt H, Gebbers J, Muller C, Arnold W, Laissue J. Human endolymphatic sac: evidence for a role in inner ear defence. ORL J Otolaryngol Relat Spec 1990;52:143–8.
[31] Harris J, Fukuda S, Keithly E. Spiral modiolar vein: its importance in inner ear inflammation. Acta Otolaryngol (Stockh) 1990;110:357–65.
[32] Tomiyama S, Harris J. The role of the endolymphatic sac in inner ear immunity. Acta Otolaryngol (Stockh) 1987;103: 182–8.
[33] Gloddek B, Harris J. Role of lymphokines in the immune response of the inner ear. Acta Otolaryngol (Stockh) 1989; 108:68–75.
[34] Yeo S, Gottschlich S, Harris J, Keithley E. Antigen diffusion from the perilymphatic space of the cochlea. Laryngoscope 1995;105:623–8.
[35] Palva T, Raunio V. Disc electrophoretic studies of human perilymph. Ann Otol Rhinol Laryngol 1967;76:23–36.
[36] Mogi G, Lim D, Watanabe N. Immunologic study on the inner ear: immunoglobulin in perlymph. Arch Otolaryngol 1982;108:270–5.
[37] Woolf N, Harris J, Ryan A, Butler D, Richman D. Hearing loss in experimental cytomegalovirus infection of the guinea pig inner ear: prevention by systemic immunity. Ann Otol Rhinol Laryngol 1985;94:350–6.
[38] Schuknecht H. The inner ear in autoimmune disease. In: McCabe B, Veldman J, Mogi G, editors. Immunobiology in otology, rhinology and laryngology. Amsterdam: Kluger Publications; 1992. p. 95–9.
[39] Harris J. Immunobiology of the inner ear: response of the inner ear to antigen challenge. Otolaryngol Head Neck Surg 1983;91:18–23.
[40] Woolf N, Harris J. Cochlear pathophysiology associated with inner ear immune responses. Acta Otolaryngol (Stockh) 1986;102:353–64.
[41] Ma C, Billings P, Harris J, Keithley E. Characterization of an experimental induced inner ear immune response. Laryngoscope 2000;110:451–6.
[42] Yoo TJ, Shea J Jr, Ge X, Kwon SS, Yazawa Y, Sener O, et al. Presence of autoantibodies in the sera of Ménière's disease. Ann Otol Rhinol Laryngol 2001;110:425–9.
[43] Cheng K, Matsuoka H, Lee KM, Kim N, Krug MS, Kwon SS, et al. Proto-oncogene RAF-1 as an autoantigen in Ménière's disease. Ann Otol Rhinol Laryngol 2000;109: 1093–8.
[44] Matsuoka H, Cheng K, Krug M, Yazawa Y, Yoo T. Murine model of autoimmune hearing loss induced by myelin protein PO. Ann Otol Rhinol Laryngol 1999;108:255–64.
[45] Yoo T, Tanaka H, Kwon S, Krug M, Yazawa Y, Suzuki M, Kitajima K. B-tubulin as an autoantigen for autoimmune inner ear disease. In: Sterkers O, Ferrary E, Dauman R, Sauvage J, Tran Ba Huy P, editors. Ménière's disease 1999 – update. Proceedings of the 4th International Symposium on Ménière's disease, Paris. The Hague, The Netherlands: Kugler Publications; 2000. p. 529–35.
[46] Moscicki RA, San Martin JE, Quintero CH, Rauch SD, Nadol JB, Bloch KJ. Serum antibody to inner ear proteins in

patients with progressive hearing loss. JAMA 1994;272: 611–61.

[47] Gottschlich S, Billings PB, Keithley EM, Weisman MH, Harris JP. Assessment of serum antibodies in patients with rapidly progressive sensorineural hearing loss and Ménière's disease. Laryngoscope 1995;105:1347–52.

[48] Disher MJ, Ramakrishnan A, Nair TS, Miller JM, Telian SA, Arts HA, et al. Human autoantibodies and monoclonal antibody KHRI-3 bind to a phylogenetically conserved inner-ear-supporting cell antigen. Ann N Y Acad Sci 1997; 830:253–65.

[49] Billings P, Keithley E, Harris J. Evidence linking the 68 kilo Dalton antigen identified in progressive sensorineural hearing loss patient sera with heat shock protein 70. Ann Otol Rhinol Laryngol 1995;104:181–8.

[50] Bloch DB, San Martin JE, Rauch SD, Moscicki RA, Bloch KJ. Serum antibodies to heat shock protein 70 in sensorineural hearing loss. Arch Otolaryngol Head Neck Surg 1995;121:1167–71.

[51] Yeom K, Gray J, Nair TS, Arts HA, Telian SA, Disher MJ, et al. Antibodies to HSP-70 in normal donors and autoimmune hearing loss patients. Laryngoscope 2003;113:1770–6.

[52] Trune DR, Kempton JB, Mitchell CR, Hefeneider SH. Failure of elevated heat shock protein 70 antibodies to alter cochlear function in mice. Hear Res 1998;116:65–70.

[53] Nair TS, Kozma KE, Hoefling NL, Kommareddi PK, Ueda Y, Gong TW, et al. Identification and characterization of choline transporter-like protein 2, an inner ear glycoprotein of 68 and 72 kDa that is the target of antibody-induced hearing loss. J Neurosci 2004;24:1772–9.

[54] Nair TS, Raphael Y, Dolan DF, Parrett TJ, Perlman LS, Brahmbhatt VR, et al. Monoclonal antibody induced hearing loss. Hear Res 1995;83:101–13.

[55] Nair TS, Prieskorn DM, Miller JM, Dolan DF, Raphael Y, Carey TE. KHRI-3 monoclonal antibody-induced damage to the inner ear: antibody staining of nascent scars. Hear Res 1999;129:50–60.

[56] Adams J. Clinical implications of inflammatory cytokines in the cochlea: a technical note. Otol Neurotol 2002;23: 316–22.

[57] Bernstein JM, Shanahan TC, Yoo TJ, Ye XJ. HLA antigens and inner ear disease. In: McCabe B, Veldman J, Mogi E, editors. Immunobiology in otology, rhinology and laryngology. New York: Kugler Publications; 1992. p. 3–11.

[58] Yeo SW, Park SN, Park YS, Suh BD, Han H, Choi HB, et al. Different distribution of HLA Class-II alleles according to response to corticosteroid therapy in sudden sensorineural hearing loss. Arch Otolaryngol Head Neck Surg 2001;127: 945–9.

[59] Melchiorri L, Martini A, Rizzo R, Berto A, Adinolfi E, Baricordi O. Human leukocyte antigen class I serum level in Menière's disease. Acta Otolaryngol Suppl 2002;(548):26–9.

[60] Lopez-Escamez JA, Lopez-Nevot A, Cortes R, Ramal L, Lopez-Nevot MA. Expression of A, B, C, and DR antigens in definite Ménière's disease in a Spanish population. Eur Arch Otorhinolaryngol 2002;259:347–50.

[61] Costa GL, Benson JM, Seroogy CM, Achacoso P, Fathman CG, Nolan GP. Targeting rare population of murine antigen-specific T lymphocytes by retroviral transduction for potential application in gene therapy for autoimmune disease. J Immunol 2000;164:3581–90.

[62] Yoo TJ, Kang AH, Stuart JM, Tomoda K, Townes AS, Dixit S. Type II collagen autoimmunity in otosclerosis and Ménière's disease. Science 1982;217:1153–5.

[63] Helfgott SM, Mosciscki RA, San Martin J, Lorenzo C, Kieval R, McKenna M, et al. Correlation between antibodies to type II collagen and treatment outcome in bilateral progressive sensorineural hearing loss. Lancet 1991;337: 387–9.

[64] Tomoda K, Suzuka Y, Iwai H, Yamashita T, Kumazawa T. Ménière's disease and autoimmunity: clinical study and survey. Acta Otolaryngol Suppl 1993;500:31–4.

[65] Yoo T, Yazawa Y, Tomoda K, Floyd R. Type II collagen-induced autoimmune endolymphatic hydrops in guinea pig. Science 1983;222:65–7.

[66] Yoo T, Yazawa Y, Floyd R, Tomoda K. Antibody activity in perilymph from rats with type II collagen-induced autoimmune inner ear disease. Ann Otol Rhinol Laryngol 1984; 93(Suppl 113):1–2.

[67] Yoo T, Tomoda K, Hernandez A. Type II collagen-induced autoimmune endolymphatic hydrops in guinea pig. Science 1983;222:65–7.

[68] Yoo T, Tomoda K, Hernandez A. Type II collagen-induced autoimmune inner ear lesions in guinea pigs. Ann Otol Rhinol Laryngol 1984;94:3–5.

[69] Berger P, Hillman M, Tabak M, Vollrath M. The lymphocyte transformation test with type II collagen as a diagnostic tool of autoimmune sensorineural hearing loss. Laryngoscope 1991;101:895–9.

[70] Hughes G, Fairchild R, Barna B. Laboratory diagnosis of immune ear disease. In: Mogi G, Veldman J, Kawauchi H, editors. Immunobiology in otolaryngology – progress of a decade. Amsterdam: Kluger Publications; 1994. p. 231–5.

[71] García Berrocal JR, Ramírez-Camacho R, Vargas J A, Millan. I. Does the serological testing really play a role in the diagnosis immune-mediated inner ear disease? Acta Otolaryngol (Stockh) 2002;122:243–8.

[72] Zeitoun H, Beckman JG, Arts HA, Lansford CD, Lee DS, El-Kashlan HK, et al. Corticosteroid response and supporting cell antibody in autoimmune hearing loss. Arch Otolaryngol Head Neck Surg 2005;131:665–72.

[73] Gray J, Ramakrishnan A, Nair S, Arts H, Telian S, Zeitoun H, et al. Predicting response to steroid treatment with serum assays in patients suspected to have autoimmune hearing loss. Assoc Res Otolaryngol. 1999, Abs.: 246.

[74] Brookes G. Circulating immune complexes in Ménière's disease. Arch Otolaryngol Head Neck Surg 1986;112:536–40.

[75] Veldman J, Roord J, O'Conner A, Shea J. Autoimmunity and inner ear disorder: an immune-complex mediated sensorineural hearing loss. Laryngoscope 1984;94:501–7.

[76] Hsu L, Zhu X, Zhao X. Immunoglobulin E and circulating immune complexes in endolymphatic hydrops. Ann Otol Rhinol Laryngol 1990;99:535–8.

[77] Harris JP, Weisman MH, Derebery JM, Espeland MA, Gantz BJ, Gulya AJ, et al. Treatment of corticosteroid-responsive autoimmune inner ear disease with methotrexate: a randomized controlled trial. JAMA 2003;290:1875–83.

[78] Sismanis A, Thompson T, Willis HE. Methotrexate therapy for autoimmune hearing loss: a preliminary report. Laryngoscope 1994;104:932–4.

[79] Sismanis A, Wise CM, Johnson GD. Methotrexate management of immune-mediated cochleovestibular disorders. Otolaryngol Head Neck Surg 1997;116:146–52.

[80] Ryan A, Harris J, Keithley E. Immune-mediated hearing loss: basic mechanisms and options for therapy. Acta Otolaryngol Suppl 2002;(548):38–43.

[81] Luetje CM, Berliner KI. Plasmapheresis in autoimmune inner ear disease: long-term follow-up. Am J Otol 1997;18: 572–6.

[82] Rahman MU, Poe DS, Choi HK. Etanercept therapy for immune-mediated cochleovestibular disorders: preliminary results in a pilot study. Otol Neurotol 2001;22:619–24.

[83] Wang X, Truong T, Billings PB, Harris JP, Keithley EM. Blockage of immune-mediated inner ear damage by etanercept. Otol Neurotol 2003;24:52–7.

[84] Cohen S, Shoup A, Weisman MH, Harris J. Etanercept treatment for autoimmune inner ear disease: results of a pilot placebo-controlled study. Otol Neurotol 2005;26: 903–7.

[85] Matteson EL, Choi HK, Poe DS, Wise C, Lowe VJ, McDonald TJ, et al. Etanercept therapy for immune-mediated cochleovestibular disorders: a multi-center, open-label, pilot study. Arthritis Rheum 2005;53:337–42.

[86] Kim N, Cheng KC, Kwon SS, Mora R, Barbieri M, Yoo TJ. Oral administration of collagen conjugated with cholera toxin induces tolerance to type II collagen and suppresses chondritis in an animal model of autoimmune ear disease. Ann Otol Rhinol Laryngol 2001;110:646–54.

[87] Parnes L, Sun A, Freeman D. Corticosteroid pharmacokinetics in the inner ear fluids: an animal study followed by clinical application. Laryngoscope 1999;109:1–17.

[88] Yang G, Song H, Keithley E, Harris J. Intratympanic immunosuppressive for prevention of immune-mediated sensorineural hearing loss. Am J Otol 2000;21:499–504.

[89] Suzuki M, Harris J. Expression of intercellular adhesion molecule-1 during inner ear inflammation. Ann Otol Rhinol Laryngol 1995;104:69–75.

[90] Takasu T, Harris J. Reduction of inner ear inflammation by treatment with anti-ICAM-1 antibody. Ann Otol Rhinol Laryngol 1997;106:1070–5.